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EXAMINER	
WOOLWINE, SAMUEL C	
ART UNIT	PAPER NUMBER
1637	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/581,814	MARCHE ET AL.	
	Examiner	Art Unit	
	SAMUEL C. WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 April 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *23-36* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *23-36* is/are rejected.

7)☒ Claim(s) *23 and 24* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *29 April 2010* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *04/29/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application.
6) ☐ Other: _____.

DETAILED ACTION

Status

Applicant's reply filed 04/29/2010 is acknowledged. All previous claims have been cancelled and new claims 23-36 have been added. Support for new claims 23-36 is found in the original claims and disclosure as filed. Since all claims are new, any new grounds of rejection set forth herein are considered as necessitated by Applicant's amendment. Any rejection not appearing below may be considered withdrawn as no longer applicable.

Information Disclosure Statement

The information disclosure statement filed 04/29/2010 fails to comply with 37 CFR 1.97(c) because it lacks a statement as specified in 37 CFR 1.97(e) or fee set forth in 37 CFR 1.17(p) (that is, either the statement or the fee was required, and neither was provided). It has been placed in the application file, but the information referred to therein has not been considered.

Drawings

The drawings were received on 04/29/2010. These drawings are acceptable Note however that the Sequence Listing submitted on the same day has been found deficient. So long as the Sequence Listing can be corrected such that the SEQ ID NOs shown in figure 5 remain valid, the drawings are acceptable.

Sequence Compliance

It is noted that the Sequence Listing supplied on 04/29/2010 has been found technically deficient (see entry on 05/06/2010 in Private Pair). An appropriately corrected Sequence Listing is required (see MPEP 2426).

Applicant is reminded to provide the necessary statement under 1.825(g) the substitute Sequence Listing introduces no new matter.

Claim Objections

Claims 23 and 24 are objected to because of the following informalities: step a in each claim recites "selected in the group consisting of". This is improper Markush claim format. The appropriate language is "selected from the group consisting of". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 23-36 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. All claims depend ultimately from claim 23 and so are rejected for at least the reasons discussed for claim 23. However, there are multiple issues involving multiple claims, and each claim will be discussed where necessary.

Claim 23: The metes and bounds of the claim are unclear. The claim recites (step b) amplifying a "long" segment of genomic DNA comprising "at least a few hundred base pairs" by multiplex "long" PCR. Note the term "long" also occurs in step

b(ii) in the phrase "for amplifying long genomic DNA segments". The word "long" is a relative term. The claim requires the "long" segment of genomic DNA to be "at least a few hundred base pairs". But how many is "at least a few hundred base pairs"? 500? 200? 150? One of skill in the art would not be apprised of the scope of the claim based on such relative terminology. If Applicant wishes to impose a minimum length for the amplified segment, the minimum length should be clear and definite (and supported in the disclosure as filed). For purposes of examination over the prior art, the examiner will presume that any segment longer than 100 base pairs is "at least a few hundred base pairs".

Furthermore, step b requires "multiplex long PCR", but only requires one pair of primers (i.e. "one or more pairs of primers") and only requires amplifying "a" long segment of genomic DNA. How could one perform multiplex PCR with only one pair of primers and by only amplifying "a" segment? The term "multiplex PCR" usually implies multiple pairs of primers amplifying multiple different segments of DNA. Applicant may wish to consider reciting step b as: "amplifying a segment of the TCRAD locus of said genomic DNA by PCR in the presence: (i) of one or more pairs of primers...". For purposes of examination over the prior art, the examiner will presume that all that is needed is the amplification of one segment of DNA with one pair of primers.

In addition, step d recites "detecting the rearranged VJ segments directly on the gel, and detecting after excitation in the UV range or at another appropriate wavelength". It is unclear whether the claim requires one detection step, or two detection steps. For purposes of examination over the prior art, the examiner will

presume the "and detection after excitation" merely refers to the manner in which detection is achieved, and not a separate, additional detection step.

Claim 24: The preamble recites "A method for the quantitative evaluation of the immune system of a human individual by genetic rearrangement of the locus TCRAD of said individual...". The underscored language seems to imply that the method involves the step of genetically rearranging the TCRAD locus. However, what is actually done in the method amounts to an analysis of genetic rearrangement of the TCRAD locus. Applicant is advised to amend the preamble to recite "by analyzing genetic rearrangement of the TCRAD locus".

In addition, the preamble of claim 24 recites "A method for the quantitative evaluation of the immune system of a human individual...as claimed in claim 23". However, claim 23 recites "A method for the diagnosis or prognosis of an immune pathology...". Therefore, the question is raised as to whether claim 24 also requires ("as claimed in claim 23") diagnosing or prognosing an immune pathology, or merely quantitative evaluation of the immune system of an individual (pathology or not). That is, the preamble for claim 23 is narrower than the preamble of claim 24. A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). As far as the steps of the two claims (23 and 24), it appears the only differences are (i) multiple segments are amplified and detected in claim 24, whereas only one segment is required in claim 23, and (ii) the detection step d of claim 24 is

more specific (narrower) than the detection step d of claim 23. Therefore, Applicant is advised to either write claim 24 with its current preamble in independent form, or write claim 24 as "The method of claim 23, further comprising..." and recite the more specific limitations of claim 24 discussed above. However, given that claims 31 and 32 each refer back to claim 24 (which currently merely recites "quantitative evaluation of the immune system"), and each of claims 31 and 32 recite more specific contexts ("for the follow-up to a treatment" and "for the measurement of the antigen receptor repertoire during the various phases of a pathology", respectively), it would seem more appropriate to write claim 24 in independent form. Otherwise, there might be similar conflict between the preambles of claims 31 and 32, and the preamble of claim 23.

Claim 24 also contains the term "long" at steps b ("multiplex long PCR") and b(ii) ("for amplifying long genomic DNA segments"). This renders the claim indefinite for the same reasons discussed for claim 23. In addition, note that claim 24 depends from claim 23, and claim 23 requires at step b amplifying a "long" segment of said genomic DNA. However, claim 24 at step b merely recites amplifying a segment of DNA (does not require it to be "long"). Therefore, claim 24 recites a broad limitation ("a segment") and (by depending from claim 23) a narrow limitation ("a long segment") in the same claim. A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Applicant is advised to avoid the use of

the word "long" and simply set forth a clear and definite minimum length if this is a desired aspect of the claimed invention.

In addition, it is unclear what is required by the phrase "for detecting, directly in a unique reaction step, several VJ rearrangements of the genes of the locus TCRAD". The limitation does not appear to have any effect here. The same step recites "amplifying a segment". But the claim later recites (step d) "the detection of the rearranged VJ segments". It is apparent from the disclosure that one may amplify multiple different segments (representing different VJ rearrangements) using one pair of primers (perhaps this is the origin of the claim term "multiplex"). If the claim requires amplifying multiple segments (which seems to be the case given the language found later in the claims, i.e. separation of fragments, detection of segments), then Applicant is advised to recite step b as "amplifying multiple different segments resulting from different VJ rearrangements of said genomic DNA by PCR in the presence: (i) of one or more pairs of primers...".

Steps c and d recite, respectively, "the separation of the gDNA fragments" and "the detection of the rearranged VJ segments". Are these even steps? A step should be recited using active language: "separating…", "detecting…". Compare with steps a and b: "extracting…", "amplifying…".

Claim 28: Also uses the relative term "long".

Claim 31: Recites "implementing the method for the evaluation of the immune repertoire, as claimed in claim 24". Claim 24 does not recite evaluating the immune repertoire. Claim 24 recites a "quantitative evaluation of the immune system".

Applicant is advised to simply recite: performing the method of claim 24 at the beginning of treatment, reiterating the method of claim 24 at various phases of the treatment, and comparing the profile of the immune system obtained each time...etc.

Claim 33: Recites that step a is carried out on a biological sample consisting of T lymphocytes of "any origin". However, claim 33 depends from claim 24, which requires the sample is selected from blood or a biopsies, so it cannot be from "any origin". Applicant may correct this by reciting in claim 33: T lymphocytes from blood or biopsies of any origin (note that claim 34, which depends from claim 33, is not inconsistent with this suggestion, since thymic cells could be a biopsy of the thymus, "other lymphoid organs" could be biopsies of those organs, etc). Alternatively, Applicant may consider amending claims 23 and 24 to strike the limitation "selected in the group consisting of blood and biopsies" and merely recite "biological sample".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 23-26, 28, 30, 33-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of GenBank GI:21363121 [online] June 10, 2002 [retrieved on October 26, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record), Wu et al (US 5,756,701, prior art of record), Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007) and Barnes (PNAS 91:2216-2220, March 1994).

With regard to claims 23 and 24, Pasqual taught a method for the quantitative evaluation of TCRAD gene rearrangement in the mouse. The method comprised extraction of genomic DNA from a biological sample (paragraph spanning pages 1164-5). The method comprised amplification of "long" segments of said genomic DNA by multiplex long PCR (paragraph spanning pages 1164-5: maximum amplicon size was ~5 kb; see also figure 1). The primers used in Pasqual's method comprised "V" primers and "J" primers (paragraph spanning pages 1164-5). Since the V gene RSS flanks the 3' end (i.e. lies downstream) of the V gene and the J gene RSS flanks the 5' end (i.e. lies upstream) of the J gene (see figure 4 of Krangel et al), Pasqual's V primer

inherently hybridized upstream of the RSS of the V gene, while the J primer inherently hybridized downstream of the RSS sequence of the J gene.

Pasqual's method used the Expand High Fidelity PCR system (paragraph spanning pages 1164-5). As evidenced by the Biochemica article, the Expand High Fidelity PCR system is a blend of Taq and Pwo polymerases, the latter having proofreading (i.e. correction) activity, the blend substantially improving elongation. Therefore, Pasqual's method inherently met this limitation.

Pasqual's amplification comprised an initial denaturing step (paragraph spanning pages 1164-5: 5 min at 94°C) and cycles of denaturation, hybridization and elongation (at 72°C, for six minutes; paragraph spanning pages 1164-5).

Pasqual's method comprised separation of amplified fragments (page 1165, first full paragraph: separation by agarose gel electrophoresis).

Pasqual's method also comprised detection of the recombined VJ segments (page 1165, first full paragraph: Southern blot of the gel followed by hybridization with probes).

With regard to claim 25, Pasqual taught using primers "specific" for given V_x and J_y genes (paragraph spanning pages 1164-5). This implicitly teaches selection of primers whose 3'OH ends are complementary only to the regions of interest.

With regard to claim 28, Pasqual performed PCR on purified (i.e. extracted) genomic DNA (paragraph spanning pages 1164-5).

With regard to claims 33 and 34, Pasqual taught T lymphocytes (T cells) from thymus (i.e. thymocytes).

With regard to claims 23 and 24, Pasqual did not perform the method on human genomic DNA (but rather, mouse genomic DNA) and did not use samples that were either blood or biopsies (the plain meaning of "biopsy" is a sample obtained from a living subject; Pasqual did not state whether the mice from which the thymus samples were taken were living or had been euthanized, although the latter is more probable). Pasqual did not perform the elongation steps of the PCR for 10 minutes. Pasqual performed only the final elongation step for 10 minutes (the claims require "steps", plural). Pasqual performed the other elongation steps for 6 minutes (paragraph spanning pages 1164-5). Pasqual did not teach detecting the amplified segments directly on the gel after excitation in the UV range or other appropriate wavelength (claim 23) of the further use of a DNA labeling agent during migration on the gel (claims 24 and 36).

With regard to claim 25, Pasqual did not teach "systematic analysis of the entire locus concerned, and in particular of the human TCRAD locus, using suitable software". Nor did Pasqual teach elimination of primers forming autodimers or stable hairpins or primers that form hybrids with one another.

With regard to claim 26, Pasqual did not teach primers selected from SEQ ID NOs: 1-21.

With regard to claim 30, Pasqual did not teach pulsed field migration.

With regard to claim 35, Pasqual did not teach amplified fragments greater than 10 kb.

With regard to claims 23 and 24, Arstila taught amplification of human complementary DNA (cDNA, i.e. not genomic DNA) for analysis of T cell receptor αβ diversity (see entire article, e.g. page 958, 2nd column, last paragraph; page 959, paragraph spanning columns 2-3). Arstila obtained T cells from blood and used these T cells to produce the cDNA for amplification (page 958, column 2: "Complementary DNA from 10^8 peripheral blood T cells from a healthy donor...".

With regard to claims 23, 24, 30, 35 and 36, Barnes taught the generation by PCR of fragments as long as 35 kb (see title) and the direct visualization of these PCR products in gels run with or later stained by ethidium bromide (a DNA labeling agent; see page 2216, column 2, "Agarose Gel Electrophoresis" and see figure 3c). In particular, Barnes taught pulsed field electrophoresis (*id.*). Note also that Barnes taught a performing the extension step of the PCR at 68°C for 11-24 minutes, which meets the limitation of claims 23 and 24, step b(ii).

With regard to claim 25, Wu taught selection of specific primer pairs and probes for analyzing specific analytes (see title). Wu taught (beginning at column 18, line 50: "PRIMER OPTIMIZATION"):

> An analysis and modification of the original primers was undertaken with the objectives of improving the amplification efficiency of each primer and decreasing the possibility of cross-reactivity among the primer pairs. Several parameters were manipulated in order to minimize physical property differences among the primers. Each primer was modified to approximately the same length, i.e., 19-24 bp. Primer oligonucleotides of this length result in greater specificity in the amplification reaction while shorter primers may result in the amplification of non-specific products. Because the efficiency of the primer pairs is also effected by the presence of hairpin loops and dimers, OLIGO 5.0 software (NBI, Plymouth, Minn., USA) was used to analyze potential primers. If hairpin loops or dimers were found, the primer sequence was modified to remove them or to, at least, diminish the effect.

With regard to claims 26 and 35, GenBank GI:21363121 disclosed the sequence of the human T cell receptor alpha/delta locus on chromosome 14. At least SEQ ID NOs: 1 and 11 (and presumably all the other SEQ ID NOs from 1-21) are found within the sequence disclosed by GenBank GI:21363121:

```
GenBank          128261  GGTCGTTTTTCTTCATTCCTTAGTCG  128286
                         |||||||||||||||||||||||||
SEQ ID NO:1      1       GGTCGTTTTTCTTCATTCCTTAGTCG  26


GenBank          989703  GTAAGTTTGAAGGGAGTGGGGGAAG  989727
                         |||||||||||||||||||||||||
SEQ ID NO:11     25      GTAAGTTTGAAGGGAGTGGGGGAAG  1
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With regard to claim 35, based upon the known sequence of the human TCRAD locus disclosed by GenBank, one of skill in the art would have expected that carrying out PCR similar to that disclosed by Pasqual would potentially generate amplicons greater than 10 kb.

It would have been *prima facie* obvious to one or ordinary skill in the art at the time the invention was made to apply the method taught by Pasqual for analyzing T cell receptor diversity to humans, using samples such as T cells obtained from blood, as taught by Arstila. One would have been motivated to do this because it was clearly of interest to those in the field to assess human T cell receptor diversity, as shown by the disclosure of Arstila. Pasqual stated (abstract): "Knowledge of the complete nucleotide sequence of the mouse TCRAD locus allows an accurate determination V-J rearrangement status." Likewise, one of ordinary skill would have reasoned that knowledge of the complete sequence of the human TCRAD locus (as disclosed by GenBank GI:21363121) would allow similar analysis of human T cell receptor V-J

rearrangements. Hence one would have been motivated to use the known human sequence to select appropriate primers for the various V and J segments, just as Pasqual did for the mouse.

Using primer design software, and choosing primers that were specific, free of secondary structure, and that were unlikely to form dimers (either autodimers or heterodimers with other primers being used) were well-known principles in the art of designing primers, as shown by the disclosure of Wu.

Pasqual's method offered an advantage over the earlier method of Arstila, in that the latter artisan's method was based on amplification of cDNA (i.e. analysis at the transcriptional level) and for only specific V and J genes. Pasqual discussed these shortcomings (page 1164, column 2, 1st and 2nd paragraphs): "Finally to date, available informations encompass essentially either analysis at the transcriptional level or gene analysis for only a few V families, thus precluding a general synthetic overview of gene rearrangements. In this report, to eliminate the biases due to transcriptional regulation...we have used a sensitive multiplex PCR assay at the genomic DNA level."

Hence one would have been motivated to supplant the approach used by Arstila with the technique of Pasqual to study human TCR diversity, to avoid bias caused by transcriptional regulation and provide a general synthetic overview of gene rearrangements.

It would also have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made in view of the disclosure of Barnes to use an at least 10 minute extension step and to use pulsed-field electrophoresis to separate the

amplified fragments; one of ordinary skill in the art would have been aware, based on the sequence of the human TCRAD locus disclosed in GenBank, of the expected lengths of the amplification products, and would have known that pulsed field gel electrophoresis was a better way to resolve such long fragments compared to standard electrophoresis. It would also have been obvious to include ethidium bromide in the gels and to visualize the products directly on the gel (as taught by Barnes) in order to dispense with the need to perform Southern blot, which would have thus saved time and expense.

Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of GenBank GI:21363121 [online] June 10, 2002 [retrieved on October 26, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record), Wu et al (US 5,756,701, prior art of record), Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007) and Barnes (PNAS 91:2216-2220, March 1994) as applied to claims 23-26, 28, 30, 33-36 above, and further in view of GenBank GI:21536269 [online] June 21, 2002 [retrieved on October 27, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21536269:OLD03:2443019 (prior art of record).

The teachings of Pasqual, GenBank GI:21363121, Wu, Arstila and Barnes have been discussed.

With regard to claim 27, while Pasqual did not analyze V, D or J segments of the TCR β chains, Arstila did analyze TCR β chain gene rearrangement by PCR with primers to specific V_β and J_β segments (e.g. page 958, 2nd column, last paragraph).

GenBank GI: 21536269 disclosed the complete sequence of the human TCR β locus.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the method taught by Pasqual for analyzing T cell receptor diversity to human TCR β chain gene rearrangements. Those of in the field were interested in such analyses as shown by the disclosure of Arstila. Pasqual stated (abstract): "Knowledge of the complete nucleotide sequence of the mouse TCRAD locus allows an accurate determination V-J rearrangement status." Likewise, one of ordinary skill would have reasoned that knowledge of the complete sequence of the human TCR β locus (as disclosed by GenBank GI:21536269) would allow similar analysis of human T cell receptor β chain V(D)J rearrangements.

Pasqual's method offered an advantage over the earlier method of Arstila, in that the latter artisan's method was based on amplification of cDNA (i.e. analysis at the transcriptional level) and for only specific V and J genes. Pasqual discussed these shortcomings (page 1164, column 2, 1st and 2nd paragraphs): "Finally to date, available informations encompass essentially either analysis at the transcriptional level or gene analysis for only a few V families, thus precluding a general synthetic overview of gene

rearrangements. In this report, to eliminate the biases due to transcriptional regulation…we have used a sensitive multiplex PCR assay at the genomic DNA level." Hence one would have been motivated to supplant the approach used by Arstila with the technique of Pasqual to study human TCR diversity, to avoid bias caused by transcriptional regulation and provide a general synthetic overview of gene rearrangements.

Claim 29 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of GenBank GI:21363121 [online] June 10, 2002 [retrieved on October 26, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record), Wu et al (US 5,756,701, prior art of record), Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007) and Barnes (PNAS 91:2216-2220, March 1994) as applied to claims 23-26, 28, 30, 33-36 above, and further in view of Liljedahl et al (US 2003/0153044, prior art of record) and Perron et al (US 2003/0198647, prior art of record).

The teachings of Pasqual, GenBank GI:21363121, Wu, Arstila and Barnes have been discussed. These references did not teach or suggest incrementing the elongation steps by 15-20 seconds per cycle.

Liljedahl taught PCR using the Expand system and stated (paragraph [0151]): "Progressively longer elongation steps are used to increase the chances of amplifying longer DNA inserts."

Perron taught PCR using the Expand system, and incremented each elongation step by 20 seconds (paragraph [0374]).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to increment the elongation steps by 15-20 seconds each cycle when practicing the method suggested by the combined teachings of Pasqual, GenBank GI:21363121, Wu, Arstila and Barnes, since this procedure was known and used in the art when amplifying long templates.

Claims 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of GenBank GI:21363121 [online] June 10, 2002 [retrieved on October 26, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record), Wu et al (US 5,756,701, prior art of record), Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007) and Barnes (PNAS 91:2216-2220, March 1994) as applied to claims 23-26, 28, 30, 33-36 above, and further in view of Dau et al (US 6,087,096, prior art of record).

The teachings of Pasqual, GenBank GI:21363121, Wu, Arstila and Barnes have been discussed. These references did not teach analyzing T-cell receptor profiles (repertoire) for the purpose of monitoring a pathology or a response to treatment in a subject, or comparing the profile of a subject to a "standard immune repertoire".

Dau taught (paragraph spanning pages 13-14):

> The ability to characterize an individual's T cell repertoire has practical applications for monitoring treatments for innumerable disorders, because the efficacy of many treatments lies in their ability to modulate (to potentiate or to suppress) an immune response. For example, when an individual is afflicted with many disorders (e.g., neoplastic disorders, chronic infection), it is desirable to provide a treatment designed to potentiate the individual's own immune response to the disorder, to suppress or overcome the disorder (i.e., it is desirable to provide an immunoproliferative treatment). A method for characterizing an individual's T cell repertoire which detects a T cell immunoproliferative response to a treatment is useful for monitoring the efficacy of such a treatment. A first characterization of the T cell repertoire as it exists prior to the treatment is compared to a second characterization of the T cell repertoire during or after the treatment to detect the presence or absence of a T cell immunoproliferative response to the treatment. Characterizations may be repeated to continue to monitor the treatment and/or to monitor for a relapse of the disorder between treatments.

Dau also taught comparing the T cell repertoire in a subject to that of a healthy human subject (paragraph spanning columns 3-4).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the method for analyzing human TCR profiles suggested by the combined teachings of Pasqual, GenBank GI:21363121, Wu, Arstila and Barnes for the purpose of monitoring response to treatment (which also represents "phases of pathology") in an individual, since it was known in the prior art as taught by Dau to use TCR profiles as an indication of response to treatment by comparing pre- and post-treatment TCR profiles, as well as to use TCR profiles as an indication of the

presence of disease by comparing an individual's TCR profile to that of a healthy individual (i.e. standard immune repertoire).

Response to Arguments

Applicant's arguments filed 04/29/2010 have been fully considered but they are not persuasive. Applicant's remarks directed to the previous rejections under 35 USC 112, 2nd paragraph, have been considered. Where Applicant's new claims obviated a particular issue under the rejection, that issue has not been raised in the current rejections. However, Applicant's amendment either did not resolve a previous issue or raised a new issue under 35 USC 112, 2nd paragraph as discussed above.

With regard to Applicant's remarks concerning the previous rejection under 35 USC 103, it is noted that the current rejection under this section has incorporated the teachings of the Barnes reference.

Applicant argues (spanning pages 10-11 of the response) that Pasqual taught obtaining genomic DNA from a concentrated source (i.e. mouse thymus) and that this step is not feasible for humans. Firstly, the examiner points out that instant claims 23 and 24 clearly require obtaining genomic DNA from a human, and instant claim 33 recites "T lymphocytes of *any origin*", with claim 34 expressly reciting "thymic cells" (which would come from the thymus). Therefore, Applicant appears to be claiming an embodiment which is "not feasible for humans". Secondly, the prior art suggested blood as a sample (which is the type of sample Arstila used as a source of T cells, as discussed in the rejections above).

Applicant also argues (and the declaration of Dr. Pasqual is largely directed to the same) that it was unexpected that a human sample could be used to produce a product that was visible on a gel. However, the disclosure of Barnes (relied upon in the rejection) clearly showed that large (up to 35 kb) fragments of DNA could be amplified by PCR and visualized directly in a gel. While this was not "human" DNA, one of ordinary skill in the art would have inferred that if DNA from a bacteriophage could be amplified and directly detected on a gel, the same could be expected of human DNA.

Applicant's arguments regarding the disadvantages of Southern blotting are noted but are moot in view of the current rejection, which provides a reason and a reasonable expectation of success in detecting such large amplified fragments directly on a gel.

Applicant's arguments on pages 12-13 pointing out what each of the supporting references teach and does not teach in not persuasive. One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicant asserts (page 13): "Accordingly, one skilled in the art could not arrive at the claimed invention through any combination of the cited references." The examiner respectfully asserts that each of the claim limitations has been accounted for by the cited combination of references.

Applicant also asserts (page 13): "Therefore, it is only through combining the specific features of the use of a human biological sample, the length of the elongation

step during amplification, and the detection step being available directly in the gel, without transfer to a membrane or the use of radio-labeled probes, that the claimed invention is possible." The examiner respectfully disagrees that the improvement of the working examples over the Pasqual reference has anything to do with whether the DNA was obtained from a mouse or a human. Furthermore, the length of the elongation step and the direct detection of the amplified product in a gel were taught by Barnes, nearly a decade before the claimed invention was made.

The arguments made in the declaration by Dr. Pasqual are similarly unpersuasive; Barnes clearly shows it was within the skill of the art to design primers, amplify a long target, and detect the target directly on a gel after electrophoresis without performing a Southern blot or using radioactive labels.

Regarding the comparative examples of the declaration (items 9 and 10), it is unclear what is different and what is the same between the two experiments. Item 9 (Exhibit B: Pasqual et al. conditions) and item 10 (Exhibit C: Instant invention conditions) both reference ADV19 and ADV20, but it is not clear if each experiment is using the same primers, the same type of DNA (mouse, human) or employ the same conditions. The cycling conditions are clearly stated for the Pasqual et al conditions: 5 min at 94°C, 26 cycles of 1 min at 94°C, 1 min at 58°C, 6 min at 72°C, and one cycle of 10 min at 72°C. But the cycling conditions used in the "Instant invention conditions" are not stated. The claims (23 and 24) recite only that the extension step is at least 10 minutes at 68-72°C. Is *this* what produces the difference in the results? Moreover, it is clearly stated that the Pasqual conditions used 1.3 unit/reaction of Expand High Fidelity

PCR system, but it is not stated what was used for the instant invention conditions. In short, because the declaration does not clearly indicate what was the same and what was different between the two experiments, it cannot be concluded whether the difference in the observed results is really unexpected. For instance, if the instant invention conditions employed a longer extension time, then it would not have been unexpected that this difference would provide a greater yield of long amplification products (see Barnes reference: figure 3c and caption; Barnes used longer extension times for longer fragments). Or for example, if the experiment for the "instant invention conditions" included the step recited in claim 29 of increasing the extension time with each repetition, an improvement would not have been unexpected given the disclosure of Liljedahl.

Furthermore, even if there are unexpected results here, in order for this to be a consideration for patentability, the claims must be commensurate in scope with the unexpected results (MPEP 716.02(d)), and the examiner has no way to assess this unless it is clear what is being compared. It would be helpful to know, for the two experiments of items 9 and 10 of the declaration:

What is being used as the template in each case (and how much)?

What are the primers in each case (and how much)?

What is the composition of the PCR reaction (concentration of dNTPs, Mg2+, enzyme, what enzyme is used in each case)?

What are the cycling conditions for each experiment?

It is also not clear what Exhibit 1C (figure 1C) is. Is it a Southern blot? Apparently it is not, but then if this is an image of a gel, why is it displayed as a negative image compared to the "poorly visible" gels in figures 1B and 2B?

For the reasons discussed above, the declaration is not sufficient to overcome the rejection.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL C. WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Primary Examiner, AU 1637